THE DEVELOPMENT OF *ONCHOCERCA VOLVULUS* IN *SIMULIUM DAMNOSUM*

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PLATES I-IV

INTRODUCTION

The mode of transmission of various species of the genus Onchocerca has been a subject for speculation and experiment for many years. In the case of *O. gibsoni* and its allies in cattle particularly, much work has been done in order to discover the means of transmission, on account of the important economic results which necessarily follow the infection of meat with worm nodules. In the case of *O. caecutiens* in Central America great attention has been paid to the severe eye symptoms which the worms in the nodules have been credited with producing. The insect-borne theory of Onchocerciasis, the water-borne theory, and the theories of soil and direct contamination have each had their supporters. On the whole the tendency is to believe that an insect-borne theory best suits the facts. The following are some of the arthropods which have been suggested or experimented with for various species of Onchocerca. Doubtless there are others, but at present it is not possible to give a complete list.

O. VOLVULUS. *Glossina palpalis, Glossina longipalpis, Pediculus capitis, Pediculus humanus, Phthirius pubis.* O. CAECUTIENS: *Simulium samboni, Simulium dinelli* suspected by Robles. O. GIBSONI: *Haematopinus vituli, Trichodectes scalaris, Haematopinus eurysternus, Culiselsa vigilax, Culicoides subnitidus, Tabanus gregarius, Tabanus nigrotarsis, Stomoxys calcitrans, Lyperosia exigua,*

Haematopinus tuberculatus, Musca domestica, Pycnosoma dux, T. cinerescens, S. elongatus, Silvius sordidus, Silvius sp., *Tabanus lineatus,* and *T.* near *circumdatus.*

The investigation of which an account is given here was commenced in 1923-24 and continued in 1925, the whole of the work being done in the Konno district of the Sierra Leone Protectorate. In the first journey in this district the presence of cutaneous larvae corresponding in morphology to those of *O. volvulus* was observed while examining scabies (craw-craw) papules. The technique used was identical with that used by O'Neill in 1875, with the exceptions that a safety razor blade did service for a scalpel and a single section was made. O'Neill was examining the papules of Craw-craw and found skin microfilaria present in numbers ; he gave a brief description of them and considered them to be the cause of this condition. The description corresponds so far as it goes with that of *O. volvulus* larvae. He describes his technique as follows :—' I find the readiest way to procure the filaria is to take between the finger and thumb a fold of the skin, so that the papule will be the highest point, then with a very sharp scalpel to slice off the epidermis, which may be discarded ; now take another slice, which will remove the base of the papule and the cutis vera.'

In the Konno district not only were the larvae present in diseased and healthy skin in many persons, but the somewhat widespread prevalence of subcutaneous nodules was noted. The fact that the larvae were not found in the blood in persons whose skin was often heavily infected, made it probable that if any blood-sucking arthropod was the vector it would be one which in the process of penetrating to reach blood would rasp or tear the skin, with the result that the larvae would be dislodged into the wound and would then be taken up with the blood.

The Congo floor maggot *Auchmeromyia luteola* appeared well-adapted to dislodge larvae in the skin on account of the damage which it does before reaching blood. It is prevalent in the Konno houses, living in the mud floor ; a number of Congo floor maggots were dissected without finding larvae in any of them.

At several villages in December, 1923, and January, 1924, it was noted that *Simulium damnosum* was biting in great numbers beside the small streams. They were a constant source of annoyance to

the boys who were then engaged in collecting snails for examination for cercariae and there was a continual slapping of legs to kill the fly, chiefly about the calves and ankles. In several cases blood could be observed trickling down the skin, especially when wet. The fact was noted, however, that after the fly had settled on the skin there was a considerable lapse of time before the person attacked felt any irritation and before the insect began to distend with blood. It appeared probable that this delay was due to the insects having some difficulty in piercing the skin, and this in turn suggested damage to the skin and the possible dislodgment of skin larvae if present. A hundred specimens of this species were dissected in order to see if any larvae could be discovered in the gut, but without any success. The investigation could not be continued at this time owing to pressure of other work.

On returning in 1925 it was determined to make a more extensive investigation in the Konno country in order to discover whether *Simulium damnosum* is capable of transmitting *O. volvulus*. The results of the preliminary blood and skin nodule investigations on the population are given here and also the results of the experiments so far carried out with a view to ascertaining the capacity of Simulium to act as vector.

A. OBSERVATIONS ON HUMAN ONCHOCERCIASIS

I. *Examination of human skin for the presence of* O. VOLVULUS *larvae.*

In proceeding north into the Protectorate, the skin of the villagers was examined in as many cases as possible for the presence of larvae and the occurrence of subcutaneous nodules. At a village called Tumbudu in the Konno district the conditions as regards skin infection with larvae, the occurrence of nodules in the subcutaneous tissue, and the prevalence of Simulium seemed favourable for the investigation and work was at once commenced here.

Agamofilaria streptocerca. This microfilaria described by Macfie and Corson in the year 1922 in natives of the Gold Coast was not discovered in the skin of the villagers at Tumbudu, where the only skin microfilaria found was that of *O. volvulus*.

Number of persons examined.

The number of natives whose skin was examined in one or more sites on the body was 123 ; of these persons fifty-two, that is over 42 per cent., had larvae of *O. volvulus* in their skin.

Fig. 1. *O. volvulus* forms in human skin.

Owing to the reluctance of females here to being examined by section of the skin, it was necessary to confine attention to males practically without exception. It is impossible, therefore, to make any comparison between the prevalence of *O. volvulus* larvae in the skin of the male and female sexes.

Number of sections of skin examined.

The total number of sections of skin examined from the 123 cases was 441 ; of these sections 102, that is over 23 per cent., were positive.

Distribution of O. VOLVULUS *larvae over the body.*

In a number of cases a more systematic examination of the body was possible ; pieces of skin were examined from four regions of the body, viz. : the scapular region, the loin, the thigh and the

ankle. Of ninety-three persons in whom this method was adopted forty-two, that is over 45 per cent., gave a positive result as regards the larvae of *O. volvulus* in one or more of the four sites. The findings in the different regions are tabulated below.

TABLE I.

Showing the distribution of *O. volvulus* larvae in 93 persons, each examined in four regions of the body.

Total persons examined	Total positive	Regions where *O. volvulus* larvae were present in the skin			
		Scapula	Loin	Thigh	Ankle
93	42	19	32	24	5

It is seen from the table that the waist region is that one of the four regions examined in which the larvae were most commonly found.

II. *Condition of skin considered in relation to presence in, or absence from, it of* O. VOLVULUS *larvae.*

The observation made in the previous tour that *O. volvulus* larvae were frequently present in portions of skin which looked perfectly healthy was fully confirmed by the present series of examinations.

Special attention was paid to the condition of the skin in sixty selected cases referred to later. It may be mentioned here that the majority of these cases were members of the more important families of the village ; that they were therefore better fed, better clothed, better washed and better housed than the average population. The condition of the skin in these cases was strikingly superior to that of the casual cases, who were often of a poorer labouring class. It was thus possible to find among these sixty cases seventeen whose skin was classed as very good, that is to say, that a complete examination of the body did not reveal any lesions of scabies, ulcers, or other obvious disease of the skin.

Of these seventeen cases having very good skins no less than eight had larvae of *O. volvulus* in sections made from various portions of the body. The youngest person having numerous larvae in a very good skin was twenty-four and the oldest fifty-one.

It appears evident, therefore, that the presence of *O. volvulus* larvae in the skin does not by any means always connote obvious

disease of the skin, even in people of advanced years ; whether this skin infection, if uncomplicated, does eventually produce obvious disease is a question which it appears impossible to answer at present.

III. *The existence of subcutaneous nodules considered in relation to the presence in, or absence from, the skin of* O. VOLVULUS *larvae.*

Sixty-eight cases were examined as to the presence of larvae in one or more skin sections, and subcutaneous nodules other than glands. The results are given in Table II.

TABLE II.

Showing the percentage of subcutaneous nodules other than glands in those having, and in those not having, *O. volvulus* in the skin.

Total examined	*O. volvulus* in skin	*O. volvulus* not in skin	Nodules present	Nodules absent	Percentage with nodules
68	30	—	12	18	40
	—	38	6	32	16

It is seen that only 40 per cent. of those having *O. volvulus* larvae in the skin had also subcutaneous nodules other than glands, while of those not having *O. volvulus* larvae in the skin 16 per cent. had subcutaneous nodules other than glands.

IV. *Condition of the lymphatic glands considered in relation to the presence in, or absence from, the skin of* O. VOLVULUS *larvae.*

The same group of sixty-eight persons was examined for enlarged glands ; in Table III are set out the figures obtained.

TABLE III.

Showing the percentage of enlarged glands in those having, and in those not having, *O. volvulus* larvae in the skin.

Total examined	*O. volvulus* larvae in skin	*O. volvulus* larvae not in skin	Enlarged glands present	Enlarged glands absent	Percentage with glands
68	30	—	8	22	27
	—	38	12	26	32

It is seen that only 27 per cent. of those having *O. volvulus* larvae in the skin had also enlarged glands, while of those not having *O. volvulus* larvae in the skin 32 per cent. had enlarged glands.

V. *The existence of subcutaneous nodules and the condition of the lymphatic glands considered in relation to the presence in, or absence from, the skin of* O. VOLVULUS *larvae.*

If we consider these sixty-eight cases from the point of view of the occurrence of either subcutaneous nodules or enlarged glands or both in cases with and without *O. volvulus* larvae in the skin, the figures given in Table IV are obtained.

TABLE IV.

Showing the percentage of persons with subcutaneous nodules or enlarged glands, or both, in those having and in those not having *O. volvulus* larvae in the skin.

Total examined	*O. volvulus* larvae in skin	*O. volvulus* larvae not in skin	Both glands and nodules	Either glands or nodules	Neither glands nor nodules	Percentage with either nodules or glands or both
68	30	—	2	15	13	57
	—	38	2	14	22	42

It will be observed that nodules or glands or both are present in 15 per cent. of those who have larvae in the skin, in excess of those who have not larvae in the skin. The margin is probably not sufficient to permit of arriving at any general conclusion as to the relationship between glands and nodules on the one hand and skin infection with *O. volvulus* larvae on the other.

VI. *The condition of the eyes considered in relation to the presence in, or absence from, the skin of* O. VOLVULUS *larvae.*

In view of the fact that profound ocular changes are stated to be due to the action of *O. caecutiens* in Central America, attention was paid to the eyes of certain cases which came for examination.

In a series of twenty-three unselected cases the vision was tested and the eyes were examined externally for evidence of disease ; in the same cases the skin was examined for the presence of *O. volvulus*

larvae. The tests of vision utilised for these illiterate natives were two, for near vision the threading of a sewing needle, and for distant vision the counting of a number of pieces of stick held at different distances. The only abnormalities found were :—slight exophthalmos without visual defect, two cases ; pterygium coupled with defect of vision approximately R 3/6 L 1/6, one case ; chronic blepharitis oedema of sclerotic and pterygium coupled with defect of vision R 3/6 L 2/6, one case ; history of discharge from eyes at intervals for one year, one case.

The results of the examination of the eyes and of the skin are set out in Table V.

TABLE V.

Showing the results of eye examination and of skin examination for *O. volvulus* larvae in 23 unselected cases.

Total examined	Skin examination		Eye examination	
	Larvae present	Larvae absent	Eyes normal	Eyes abnormal
23	11	—	7	4
	—	12	11	1

At first sight it might appear that there were more abnormal eyes among persons having *O. volvulus* in the skin than in those not having these larvae in the skin. It appears possible, however, that this apparent difference may to some extent be explicable on the ground of age. The persons who had larvae in their skin in this series happened to be much older than those who had no larvae in the skin. Thus the average age of the seven having normal eyes and larvae in the skin was forty years and that of the four having abnormal eyes and larvae in the skin was forty-seven years ; whereas the average age of the eleven having normal eyes and no larvae in the skin was twenty-six and the age of the case having abnormal eyes and no larvae in the skin was twenty-five.

A series of thirty-eight cases selected in age periods gave a different result as seen in Table VI.

TABLE VI.

Showing the result of eye examination and of skin examination for *O. volvulus* larvae in 38 selected cases.

Age	Total examined	Skin examination		Eye examination	
		Larvae present	Larvae absent	Eyes normal	Eyes abnormal
	38	18	—	18	0
	—	—	20	19	1
0 to 10	8	0	—	—	—
		—	8	8	0
11 to 20	3	1	—	1	0
		—	2	2	0
21 to 30	9	6	—	6	0
		—	3	3	0
31 to 40	6	3	—	3	0
		—	3	3	0
41 to 50	5	4	—	4	0
		—	1	1	0
51 over	7	4	—	4	0
		—	3	2	1
TOTAL ...	38	18	20	37	1

In this series only one case had abnormal eyes, a condition of pterygium with history of discharge from the eyes periodically: vision good.

No evidence of such conditions as irido-cyclitis, keratitis punctata, or panophthalmitis was obtained in either of these series. On the contrary the general appearance of the eyes and the visual acuity even in the older cases was excellent. In this locality, therefore, it was not possible to obtain evidence which would justify any etiological association between disease of the eyes or defects of vision and the infection with *O. volvulus* as determined by skin and nodule examination.

VII. *Character of the subcutaneous nodules.*

These varied from a smooth, soft, just palpable nodule to a large, irregularly nodular, hard mass of the size of a large walnut. The first type was usually found in the region of the great trochanters, on the ribs (Case 50), and on the scalp ; the second type was generally closely associated with joints, more especially the elbow and knee joints ; neither type was usually adherent to the skin, but in some cases, especially over the great trochanter region, the skin over the nodule was thickened, partly adherent, and polished on the surface from pressure.

Pain was frequently complained of, and some persons who had nodules on both trochanter regions had resorted to the use of a circular ring-pad resembling a magnified bunion pad to enable them to sleep on the side.

Cases were examined in which larvae were found in the skin without palpable nodules being present ; cases were found where *O. volvulus* larvae were present in the skin while the nodules which were present gave negative results on puncture ; cases were found where *O. volvulus* larvae were present in the skin and also in nodules ; there were no cases in which, although no larvae were found in the skin, puncture of a nodule gave a positive result.

Juxta-articular nodules.

If the region had not been one in which *O. volvulus* was present, several of the cases seen would have been classed as juxta-articular nodules.

The examination of puncture fluid by no means always cleared up the diagnosis. In this connection special reference may be made to two cases.

Case 59. This was a man of fifty-five years of age, whose skin was apparently normal except over the knees and elbows, where it was much wrinkled ; the eyes were free from disease and the visual acuity was good ; the blood was negative. On both sides the inguinal glands were enlarged. He had the following nodules :—

Near	R trochanter	...	2 each about	$1\frac{1}{2} \times 1\frac{1}{2}$ inches
	L ,,	...	2 ,,	$1\frac{1}{2} \times 2$,,
	R elbow	...	1 ,,	2×2 ,,
	L ,,	...	1 ,,	2×1 ,,
	L knee (head of Tibia)		1 ,,	1×1 ,,

These nodules were irregular in shape and had accessory small nodules at the margins. They were hard, fairly mobile under the skin, and non-adherent. They gave the impression of resulting from the fusion of several nodules.

Puncture of the left elbow nodule resulted in a flow of amber-coloured fluid oozing slowly from the needle. Puncture of one right trochanter nodule produced a few drops of serous fluid ; the puncture of the remaining nodules gave small amounts of an opalescent fluid with light brown granules in it. In addition to each nodule being punctured once, two of the nodules, one on the right trochanter and one on the left trochanter, were punctured a second time in a different place. None of these nine punctures revealed the presence of either eggs or larvae. It would perhaps have been legitimate to conclude that these nodules were not connected with infection with *O. volvulus*. Such a conclusion, however, appeared less justifiable in view of the fact that the examination of skin sections revealed a particularly heavy infection with *O. volvulus* larvae in the scapular, loin, and thigh regions, although not in the ankle region ; gland puncture was not permitted.

Case 51a. Here the nodules were not so numerous but they were of a similar character and gave an even more interesting result. The nodules were :—Right femur internal condyle, two ; right trochanter region, two. Puncture of the four nodules gave a negative result for three and a positive result for one, namely, one of those over the condyle of the femur. The blood was negative, but the skin of scapular, loin, thigh and ankle regions was heavily infected with larvae of *O. volvulus*.

B. OBSERVATIONS ON *SIMULIUM DAMNOSUM*

A. General.

Hour of day at which biting.

Observations were made at various hours of the day in order to ascertain at what hours the fly was prepared to feed in this locality and whether any particular hours were preferred by it. It was found that individuals were not captured biting earlier than 6 a.m. and that only the latest stragglers were biting as late as 6 p.m. In the

middle of the day the fly would not go far in order to bite, not even five yards as a rule. The flies were found biting, however, in bright sunny weather at any hour of the day, provided they had only a yard or two to go from their shelter. Even on sunny days they were thus captured at any hour of the day from 6 a.m. to 6 p.m. On dull days without rain they bit freely at any hour of the day and a slight shower did not reduce the catch materially. On wet days, however, with heavy rain, they were not found biting, and it was noted also that soon after a heavy rain there was a considerable lapse of time before they would again come out to bite. All these records were made close to the edge of water, usually flood water from the large river or near small streams.

It was frequently noted that during a period which gave a good capture there were intervals in which the fly was not biting. They thus appeared capricious at times when no alteration was noticeable in the temperature or light ; the fly was sought out in places where it was known to exist and the collectors encouraged them to bite by disturbing their resting place in the long grass and bush and submitting quietly to their attacks. It was noticeable that the fly did not come out to attack as it was observed to do in the month of December in another locality.

Length of life in captivity.

Flies captured while just commencing to feed and kept in tubes of various sizes without food, and with or without moisture supplied, whether kept in the dark or in the light, failed to survive more than three days.

Various experiments were made in order to discover some means of keeping wild flies alive in captivity, but the results were disappointing ; the containers were covered in each case with gauze of 50-55 meshes to the inch ; this, in the case of solid containers such as those of tin and wood, made the inside dark. The containers used were of glass, metal or wood, and were of various sizes and shapes. Foods of various kinds, e.g. moist raisin, fresh banana, oranges, and particles of meat were supplied ; the condition of moisture was varied by adding damp blotting paper, damp sand or free water. Efforts to reproduce the conditions of nature consisted in placing small plants with the soil in which they were growing in several of the containers.

In spite of the efforts made, the longest period of survival in a total of 268 flies was ten days, and that a solitary instance. Individual specimens lived for eight and seven days, but by far the majority were dead within four days. On the whole, it may be said that dry glass cylinders and large test tubes with raisin as food gave better results than did either wood or metal containers, but the cases of survival were few and far between.

Effects of sunlight.

Fully-fed flies exposed in test tubes immediately after the feed to the full glare of sun just before noon died in twenty to sixty seconds after a period of violent excitement. Partially-fed flies survived for one to two minutes.

Feeding in captivity.

In spite of numerous attempts made, using various expedients such as moistening the skin, smearing with plant juices, serum or blood, no fly was ever induced to bite the human skin, either white or black, in captivity. Flies were observed to sit on moist raisin and appeared to be attempting to feed, but they did not engorge ; they apparently took up a little water from time to time from the muslin at the edge of the moist food.

Time taken to feed and attitude in feeding.

1. *On human beings.* The time required by the fly to feed to repletion was noted on several occasions, when flies were observed to settle and bite and fly away unmolested. It was found that the time varied from one-and-a-half to five minutes. There is a considerable interval after the fly alights and begins to operate on the skin before any distension of the abdomen is visible ; in flies captured at this point and dissected, the gut frequently contained cellular débris ; the majority of the time was occupied in getting down to the blood level and after this was reached distension was a rapid process. The attitude at the commencement was with the body parallel to the skin surface, the wings flat and almost overlapping ; by the time the abdomen was nearly distended the position was altered so that the whole body was at an angle to the skin with the head close down to it and the tip of the abdomen well raised from the surface.

They were easily disturbed in the early stages of their feed, but after they had reached blood and commenced to engorge, they were not disturbed by placing a test tube over them and went on feeding till distended, and they were thus easily captured. The extent to which these flies engorge is remarkable and the abdomen distends visibly owing to the large accumulation of blood in the mid-gut ; in specimens dissected at periods varying from half-an-hour to

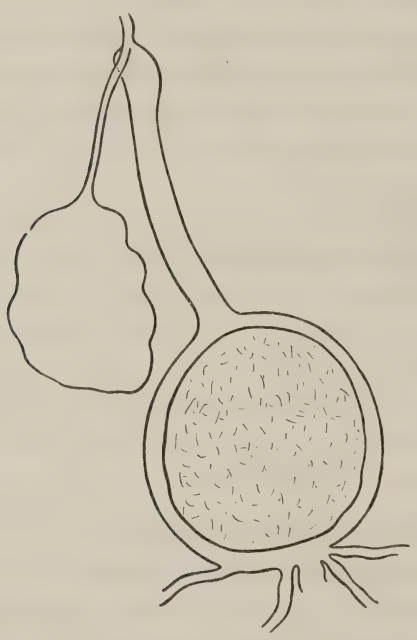

Fig. 2. Midgut of *Simulium damnosum* distended with human blood.

six hours after feeding, the blood was always found in this organ and never in the thin membranous structure which apparently corresponds to the food reservoir of tsetse-flies. The blood forms a globular mass which retains its form even after being pressed out of the cut anterior end of the gut ; the blood mass is semi-solid, sticky in consistency, and difficult to disintegrate by needles, soon after the feed ; altered blood was found in small amounts up to four days after feeding. The food reservoir was usually found to be full of a clear glairy fluid after the expression of which the thin walled sac collapsed.

2. *On animals.* Only a few experiments on animals were made, employing a sheep, two young goats, and a dog. These were taken at various times to the spots where flies were being captured biting human beings. The fly was not seen to bite the animals when these were walking about. It was possible, however, by placing the young goats on their backs to observe that two flies bit them and engorged with blood in the regions of the mammary gland and axilla. The time taken to engorge did not exceed the time found for human beings, and the degree of engorgement was the same.

Effects of rain and immersion in water during feeding.

It was found that a coarse spray of water applied to the fly while biting caused it to relinquish its hold. In the same way the direct impact of rain drops on the feeding fly dislodged it. If, however, the limb on which the fly was feeding were immersed gently in water the fly very frequently did not relinquish its hold.

1. Fly biting on leg just above ankle ; whole foot and ankle gently immersed in still water so as to cover fly to a depth of two inches ; fly remained and continued biting under water and swelled out ; on removing foot gently the fly was still attached but almost immediately flew off.

2. In a similar experiment, the fly remained immersed for five minutes but was loose on bringing the foot to the surface.

3. A feeding fly was immersed for three minutes ; on removal of the foot was still feeding.

4. A fly biting on dorsum of foot was immersed ; in three minutes came up and stuck to hairs on leg just below surface of water ; thirty seconds later it suddenly shot up to the surface, circled about rapidly for a few seconds on the surface and then flew off.

The fly appeared to have attached to it bubbles of air when it came up to the surface, and it made rapid gyrations on the surface, seemingly until these bubbles burst, before flying off.

Cutaneous and general reactions after bites.

When a fly settles, which it does without sound, and commences biting, nothing is noticed as a rule if the attention is otherwise occupied ; in a large number of experimental observations the

person being bitten was quite unaware of it, even when the fly was engorging with blood, if the attention were otherwise engaged; in other cases where the attention was directed entirely with the object of detecting when the fly was biting the initial stages of the bite were not noticed, and irritation was first felt when the fly commenced to draw blood.

Certain persons, few in number, failed to observe that they were being bitten even when their attention was directed to detecting bites, and the flies would engorge and go off unperceived. There is great variation in sensibility in this respect, but when flies are biting in any number even the most unobservant becomes aware of it.

The first thing noticed is a very slight scratching sensation which goes on for about a minute or more; this is followed by a slightly painful pricking sensation and this finally by an intense irritation which comes on just as the fly is ready to move away.

If the affected part is immediately scratched the irritation quickly passes off, the lesion made by the fly having been enlarged by the scratching. In natives, even if scratching was prevented little local reaction could be seen on the skin; they appeared to be immune; on the skin of a white person examined, the reaction on the unscratched skin consisted of a white raised wheal about 5 to 8 mm. across with a red point in the centre; intense irritation was felt for ten minutes, and subsequent slight irritation for about two days.

In cases where numerous flies bit close together on the skin, especially when it was damp, the lesions would occasionally ooze somewhat freely and the blood droplets would coalesce and form a drop which trickled down. Non-biting flies were seen to settle on the skin and feed on the blood in such cases.

Prevalence of fly.

Some indication of the numbers of fly actually biting when given the opportunity in their haunts at this season may be gained from the following figures; each number given represents those caught on a single individual; no males were captured.

On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 73
On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 58
On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 55
On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 10

On 31.7.25, bright and sunny, 8 a.m. to 2 p.m.	1 boy caught 80
On 1.8.25, dull, sunless, 7.30 a.m. to 8.30 a.m.	1 boy caught 25
On 1.8.25, dull, sunless, 10 a.m. to 4 p.m.	1 boy caught 46
On 24.8.25, sunny, 8 a.m. to 10 a.m.	1 boy caught 24
On 25.8.25, dull afternoon	1 boy caught 30
On 25.8.25, dull afternoon	1 boy caught 30

It is seen that here we have a fly which is so numerous and bites so freely that any individual during the course of the day may receive a very large number of bites. Even although only a small percentage of those which bite acquire or transmit a disease virus it is clear that the very large numbers would make the transmission probable, provided that subsequent biting occurs after such an interval as would permit of the development to an infective stage of the virus in the fly.

Situation on the body of bites received.

In considering the part of the body bitten it may be stated that the experiments were carried out in those places where flies were being captured, that is, in fairly open areas without much high shade and in grass about three to six feet high with a few shrubs among it. A small portion of the low grass was beaten down and the person stood or sat in the clearing. It was found, if a person stood erect with feet, legs, and thighs exposed to bites in such open situations, that about 80 per cent. of the total bites were received below the level of the knee. The fly attacked a portion of the skin which was in shade and a favourite site was just below the bulge of the calf or below the malleoli. When the person sat or squatted in the cleared area with the skin exposed, the buttocks and loins were chiefly attacked ; a person standing among the grass before it was trampled down was bitten wherever the grass reached on his body. The fly did not, as a rule, leave the shade afforded by the grass and the lower parts of the trunk, and attack the face, neck and shoulders. It was observed that while sitting on a camp-stool in the centre of the small cleared area, the arms, neck and face, although exposed, were never bitten during an hour, while persons standing were bitten below the knee and those sitting on the ground were bitten on the loins, buttocks, and legs.

Habits as regards following human beings.

Native accounts describe this fly far from water and pursuing the rice harvesters on dry farms; little evidence of following was obtained however, during the period of investigation. The nearest point to the rest-house at which fly could constantly be captured was 150 yards away. There was a steady traffic of persons to and from this spot at the edge of the river, yet in the whole period only half-a-dozen flies were caught biting in the rest-house compound. Apart from the fact that persons passing through the position occupied by the fly were not followed, there is also the fact that the bush extends from the spot right up to the rest-house compound. It is seen, therefore, that little or no movement of the fly through the bush was taking place here at this time. In experimenting on this question it was found that if the fly-collector was standing or sitting in a small cleared area at the edge of the long grass, other persons could stand on the path at a distance of only five yards from this spot and hardly ever receive a bite; at two yards the bites were more numerous, more especially if the skin was moistened with water. There was thus no evidence here at this period of the year of following of human beings by the fly from those of its permanent sites, near the water, which were tested.

Types of country in which fly was found biting.

The proximity to water was the essential feature noted. In Plate II, fig. 3, is shown a river of some size on the shelving banks of which, where they were grass-covered and provided with shade from trees and shrubs, the fly could be found biting at almost any hour of the day except in actual rain or immediately after a heavy rain.

On the opposite side (Plate II, fig. 4) of this river the banks are low, and overflow was constantly occurring with each rain. Among the grass at this side the fly was constantly to be found also. The collector simply walked in from the path at any point, trod down some of the grass, beat the standing grass with his hands and, taking off his clothes, sat down. In a few minutes dozens of flies would be round him trying to select a shady spot on his skin on which to feed.

The wet rice farms also were favourite spots ; most frequently these lay on each side of small slow streams and here the rice was growing on sodden ground. There might, or might not, be overhead shade, but if not, the fly bit very low down. Some collectors found that by first wading through water and then offering their skin to the fly, they received more bites.

Habits of people in relation to bites of Simulium.

People bathing in the rivers are not frequently bitten, but those who stand on the banks of the river suffer severely. The farming operations involve both sexes ; the bush is cleared by the men, a certain amount of weeding is done by the women and both take part in cutting the rice. This process involves walking through the growing rice and cutting the tops off, the straw being trampled under foot. The clothing worn in these operations is reduced to a loin cloth, or this and a very short shirt may be worn. The places on the body which would therefore most usually get bitten are the regions below the level of the top of the growing rice, that is, from the waist downwards. Young children are employed to frighten the birds, but are perched on high pedestals erected on the farms.

In the Konno country all go to the waterside to defaecate. The waterside area is in close bush and the latrine is normally the edge of a small stream. Here the fly has an excellent and frequent occasion to bite about the waist region of both sexes. Both the habits of the fly as seen here and the habits of the people tend to produce the same effect, namely, that it is the region from the waist down that is most attacked ; possibly males are more exposed to its attacks than the females during their farming operations.

Native names.

Some of the tribal names for Simulium in Sierra Leone are :—

Konno	...	Mooli or Mooyee
Timne	...	Mapus
Mendi	...	Mawwee
Lokkoh	...	Poondee gootena, i.e. short mosquito (Poondina = mosquito).
Susu	...	Koondee
Mandingo	...	Mootee

B. DISSECTION OF WILD SIMULIUM

Gut of fly.

Boys were taken to places where the fly had been observed biting and instructed to catch each fly after it had begun to feed on the skin. The flies were then dissected in order to see whether any of them were ingesting *O. volvulus* larvae, the gut alone being examined. Of a total of 780 flies which were so dissected twenty, that is, 2·6 per cent., had in their gut larvae which appeared in the fresh state to be *O. volvulus* larvae, and which on staining showed the morphological characters of this larva. The larvae were conspicuously active in the gut contents of the fly, much more so than when dissected out of pieces of skin in a drop of water on a slide. The blood was found soon after a meal to be agglomerated into an amorphous-looking, spherical mass, and the larvae could be seen actively moving chiefly between the anterior surface of this mass and the wall of the mid-gut ; they were active in flies which had died six, twelve, and even eighteen hours before dissection.

It was, therefore, proved that Simulium is capable of taking up, in feeding, larvae having the character of the larvae of *O. volvulus*, and further, it was proved that the larvae, having been ingested, not only retained vitality but definitely increased in the activity of their movements ; the latter fact pointed to the possibility of the larvae undergoing further development in the fly.

Thorax of fly.

Flies which had been captured in the same manner by the boys were examined as to the occurrence of developmental forms of larvae in the thorax. Of 1,320 flies in which the thorax was dissected developmental forms of larvae at different stages were found in fifteen, that is, over 1 per cent. This investigation showed that nematode larvae of some kind were capable of development in the thoracic tissues of Simulium.

Head of fly.

This was dissected in the case of 1,140 flies caught by the boys ; in no case was a larval form found actually in the head, although one form was partially embedded in the head and was pulled out of the anterior thoracic region with the head.

Identity of the thoracic forms.

While, therefore, there was evidence that development of some larvae was capable of proceeding in Simulium there was no evidence that the developing forms were part of the life-cycle of *O. volvulus ;* the only suggestive points were that, as previously stated, it had been proved that Simulium could readily take up larvae morphologically identical with this in feeding, and that when in the insect's gut the larvae showed increased activity and good viability.

In order to obtain confirmatory evidence bearing on this point the following examinations were carried out, the object being to determine as far as possible by a process of exclusion what the larvae were which were present and developing in the thorax of wild Simulium in this district.

I. Examination of day and night cutaneous blood of selected human beings.
II. Examination of day cutaneous blood of unselected human beings.
III. Examination of day cutaneous blood of domestic animals.
IV. Examination of the skin of domestic animals for microfilariae.

Examination of day and night cutaneous blood of selected human beings.

Sixty persons, all males, were examined for microfilariae in the cutaneous blood of the ear at noon ; a thick film for each person was examined. It was intended to examine the same persons at midnight, but there was some difficulty about persuading them, so that only forty-six of them could be collected for the midnight examination.

The cases were selected so as to give ten persons in each of six age groups and the results obtained are set out in Table VII.

A single sheathed microfilaria present at noon in the film of one individual in the 41-50 age group proved to be *Microfilaria bancrofti,* the same individual at midnight being found to have numerous microfilariae of this species present in the cutaneous blood. All of the other persons who had microfilariae in the cutaneous blood at midnight were also infected with this parasite, no other being found.

This examination, therefore, revealed no infection with either

TABLE VII.

Showing the findings in the cutaneous blood of 60 males at noon and 46 males at midnight.

Age period	Noon		Midnight	
	Number examined	Number having microfilariae	Number examined	Number having microfilariae
0 to 10	10	0	10	0
11 to 20	10	0	3	0
21 to 30	10	0	9	4
31 to 40	10	0	7	2
41 to 50	10	1	8	3
Over 50	10	0	9	4
TOTALS	60	1	46	13

Acanthocheilonema perstans or *Loa loa*, while it demonstrated a moderate infection of this sample of the population with *F. bancrofti*.

Examinations of additional persons at a late hour of the night were not possible owing to the reluctance of the villagers to attend. Additional cases were, however, examined at various hours of the day from soon after 6 a.m. to nearly 6 p.m., as shown in the following Table.

TABLE VIII.

Examinations of day cutaneous blood of 83 unselected human beings.

Time	Number examined	Infected with microfilariae
6 to 7 a.m.	4	0
7 to 8 a.m.	9	0
8 to 9 a.m.	7	0
9 to 10 a.m.	9	0
10 to 11 a.m.	11	0
11 to 12 noon	21	0
12 to 1 p.m.	5	0
1 to 2 p.m.	5	0
2 to 3 p.m.	5	0
3 to 4 p.m.	4	0
4 to 5 p.m.	2	0
5 to 6 p.m.	1	0
	83	0

It is seen that in this series also neither *A. perstans* nor *Loa loa* was found.

As a result of this examination it appeared improbable that the larva found developing in the thorax of Simulium was that of either *A. perstans* or *Loa loa*. It might be that of *F. bancrofti*, as the popula-

tion harboured this parasite in a fair proportion of cases. Against this, however, we have the biting habit of the fly, which was found biting here at this season only between the hours of 6 a.m. and 6 p.m. It appears possible, however, that the fly might become infected with this parasite even if its biting was entirely diurnal, because *F. bancrofti* larvae were seen once at mid-day in one case and possibly were present in the cutaneous blood of other cases in numbers which, although too small to be revealed by the examination of a single thick film, might yet be taken up by the fly in biting.

III. *Examination of day cutaneous blood of animals.*

No cattle or horses were present in the village of Tumbudu nor were any seen at any of the sites at which the boys were engaged in collecting flies.

There were a few sheep and goats and one dog in the village, and blood from the ear of the majority of these was examined in fresh film. Thirty-one sheep and nine goats were examined in this way without the discovery of any microfilariae in them. An examination of these animals was all the more necessary as it was proved that *Simulium damnosum* is capable of engorging upon goats at least.

IV. *Examination of the skin of animals for microfilariae.*

The portion of the tip of the ear of each animal from which blood was being taken was kept and teased up with needles in a drop of water on a slide and examined for microfilariae ; in no case was a microfilaria found.

The general effect of the results of these examinations was to give the impression that whatever the larva was which was found developing in the thorax of wild *Simulium damnosum*, it was probably neither that of *A. perstans*, *L. loa*, or *Ag. streptocerca*, nor of a filaria of cattle, horses, goats, or sheep. The probabilities rested then between *F. bancrofti* and *O. volvulus*.

One of the direct results of this series of examinations was that it was rendered possible to make an advance by attacking the problem in a somewhat different way. There now became available certain persons of whom it was known that they did not present

larvae of *L. loa*, *A. perstans* or *F. bancrofti* in their cutaneous blood, and in whose skin only *O. volvulus* could be demonstrated. The cases which fulfilled these conditions were re-examined and two of them were selected as presenting the most intense infection of the skin with *O. volvulus*. These cases were then used to replace the previous fly-collectors ; they were taken to the chosen site and each was attended by a boy whose work consisted in collecting flies off one of these two cases. The process of collecting was carefully supervised in order to avoid the possibility of the flies being captured on any other person than the two special cases.

The immediate effect of adopting this procedure was that whereas the gut infections had hitherto been 2·6 per cent. in 780 wild flies caught on the unselected boys engaged in collecting, it rose at once to 17 per cent. when the flies dissected were all collected from these two cases. At this stage the less heavily infected case was discarded and the remaining case was alone exposed to the bites of the fly, and in successive experiments this man was clothed so as to allow only certain areas of skin to be bitten.

First of all, he was clothed so as to expose to the fly only the region from the loins upwards ; as a result of this the percentage of fly infection in the gut rose to 28. In the next experiment all the body was protected except the waist and hips; flies captured feeding on this area showed a gut infection percentage of 59.

Finally the case was exposed to the fly in such a way as to allow biting only on a narrow band of skin four inches wide round the hips, having the subcutaneous nodules, which were present near each trochanter, in the centre of the band. In this last experiment the percentage of gut infected flies rose to 80.

The results are shown in Table IX ; the fact that the area of body exposed was more and more restricted necessarily had the result that smaller numbers of flies could be collected from the case. The blood of these cases was examined frequently, usually just before starting out to collect and immediately on return. No blood microfilariae were found other than, on two occasions, *Microfilaria volvulus* itself.

TABLE IX.

Showing increase in percentage of gut infection in *Simulium damnosum* by allowing it to feed on selected cases and on special areas.

Feeding on	Number of flies	Gut infections	
		Number	Percentage
Unselected boys	780	20	2·6
Cases 47 and 50 (whole body)	41	7	17·0
Case 50 (loins upwards)	54	15	28·0
Case 50 (waist and hips only)	29	17	59·0
Case 50 (on band of skin round hips with nodules in centre)	20	16	80·0

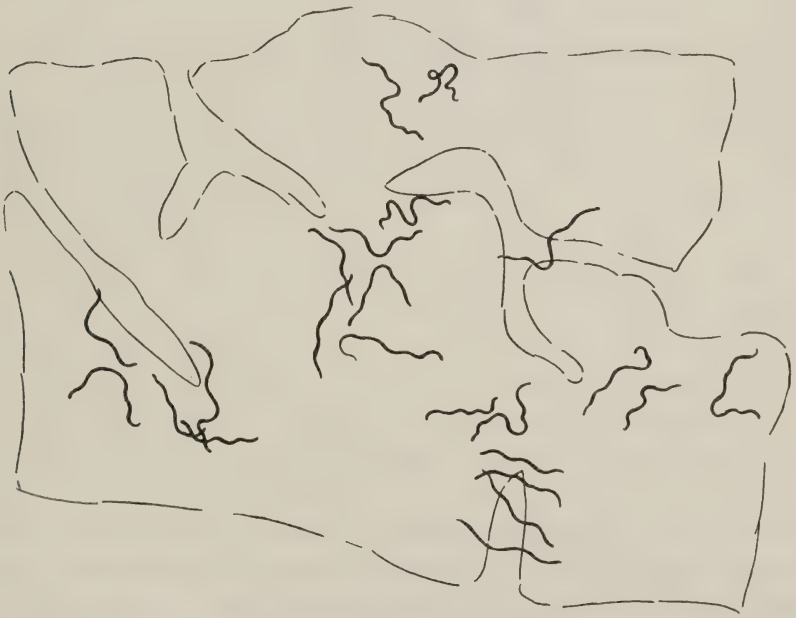

FIG. 3. Edge of blood mass in anterior part of midgut of *S. damnosum*, with larvae of *O. volvulus*.

This series of experiments not only served to confirm, amply, the observation that Simulium can take up the larvae of *O. volvulus* from the skin, but also showed that when selected areas of heavily-infected skin were exposed to it very large numbers of larvae were ingested. In some flies between one and two hundred *O. volvulus* larvae were counted in the gut. It seems probable that when this species of Simulium feeds on an area of skin affected with *O. volvulus*

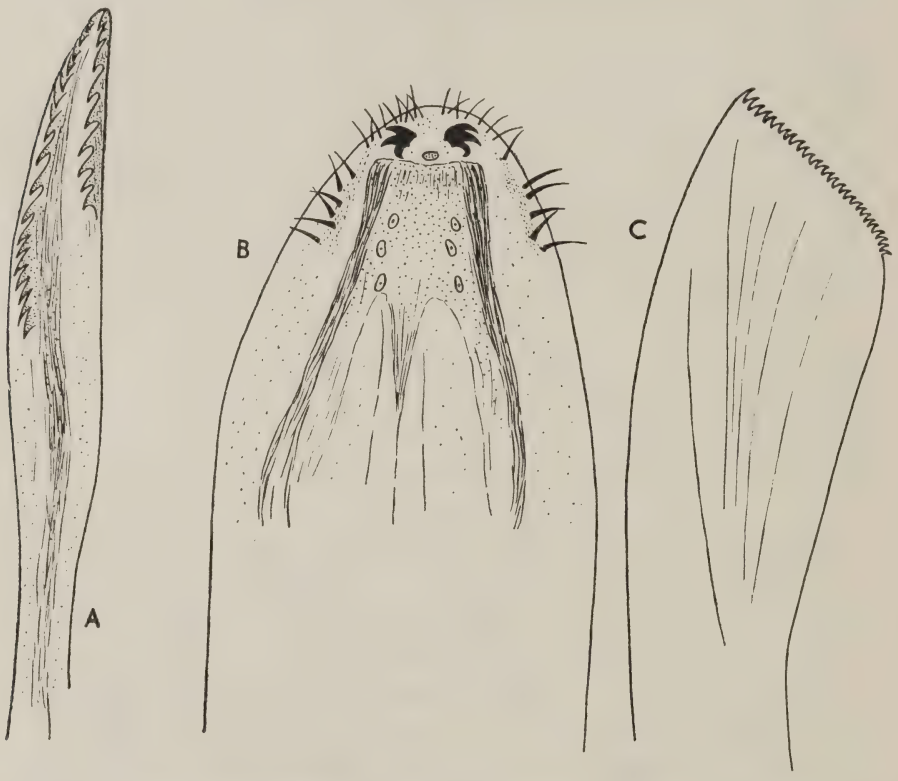

FIG. 3A. *Simulium damnosum.* A.—Maxilla; B.—Labrum epipharynx; C.—Mandible.

larvae it invariably takes up some of them. Corroboration of this view is found in the study of the mouth parts and the ragged type of lesion which the biting parts of this species inflict on the skin.

At the same time the experiments demonstrated the fact that it is not necessary that the larvae of *O. volvulus* should be circulating in the blood in order to permit of their being taken up by a blood-

sucking insect. The repeated examination of the cutaneous blood of these cases during the period proved conclusively that the larvae were very rarely found in a drop of blood taken from the skin ; even on the occasions on which they were found in such a drop it was not feasible to exclude the possibility of their having been derived from the skin ; in fact, that this was the source of those larvae appears highly probable.

Development of O. VOLVULUS *larvae in Simulium.*

The next step was to endeavour to ascertain whether the *O. volvulus* larvae which were so readily and constantly taken up by *Simulium damnosum* would proceed to develop in the body of this insect provided it was kept alive after feeding on infected skin. Incidentally it would be of interest to discover whether the developmental forms, if found, would correspond to those forms which had already been found in a percentage of the wild flies captured biting on unselected persons. The experiments were as follows :—

(1) Ten flies which had fed on a boy infected in the loin skin, but which were collected from any portion of his body, were kept until they died and were dissected. In one fly which died after thirty hours from the time of feeding, development forms of an early stage were found in the thorax. The other nine, which had all died before three-and-a-half days, proved to be negative.

(2) Large numbers of flies were captured on the two cases (47 and 50) feeding on any part of the body. Of the total of flies so captured very few survived more than a few days. Twenty-two, however, were dissected soon after their deaths and in this total, seven, i.e., 32 per cent., proved to be infected in the thorax.

(3) Twenty-two flies which were captured biting on Case 50, on the band of skin four inches wide, with the nodules in the centre, as described previously, were kept alive. Of these twenty-two flies, which were all dissected at periods later than two days after their feed, eighteen, or 82 per cent., proved to be infected in the thorax. In Table X are set out the percentages of thorax infection obtained in these experiments.

TABLE X.

Showing increase in percentage of thorax infection in *Simulium damnosum* by allowing it to feed on selected cases and on special areas.

Feeding on	Number of flies dissected	Thorax infected	
		Number	Percentage
Unselected boys	1,320	15	1·1
One infected boy (all over body)	10	1	10·0
Cases 47 and 50	22	7	32·0
Case 50 (nodule band)	22	18	82·0

From a consideration of these experiments on gut and thorax infection it appeared that when flies were given an opportunity of feeding on known infected skin, the percentage of gut infections rose to a very high figure, such as was never approached by flies captured on casual collectors; again, such flies when kept alive showed a percentage of infection with developing forms in the thorax which was far in excess of anything obtained from the dissection of wild flies fed on casual collectors. In view of the process of exclusion of other larvae, both human and animal, which preceded the last part of this investigation, there appears no doubt that the forms which were developing in the thorax of such flies as fed on the selected cases were derived from larvae ingested from the skin of these cases by the fly and that they were, in fact, the developmental forms of *O. volvulus*.

Examination of other insects.

Reference has already been made to the dissection of the Congo floor maggot. *Glossina palpalis*, which was present but not plentiful in the bush adjacent to the Moendi River, near Tumbudu, was captured on five occasions when feeding on Case 50. Of these five tsetse-flies, two had larvae of *O. volvulus* in the gut, one fly having one and another fly having two. The flies were dissected within an hour of feeding but the larvae were motionless and appeared to be dead. From these few specimens it is evident that *G. palpalis* is capable of taking up the larvae of *O. volvulus*, but it does not appear

that it can take up any numbers comparable with those taken up by Simulium. Further, whereas the larvae in the gut of *Simulium damnosum* became more active, the reverse was the case for the larvae discovered in the gut of the two infected tsetse-flies.

Tabanus sp.*

Forty-seven specimens of this fly were dissected in the gut and thorax without the discovery of any larvae. These flies were very common at this place but the natives were seldom bitten by them while they were under observation. The fly is conspicuous not only by its size but by the formidable sound which it makes, and before it settled on the skin the natives were generally prepared for it and dealt with it immediately.

Development of ovaries in Simulium fed on human blood.

The condition found at the time of capture is shown in fig. 4, the ovaries appearing as purely abdominal organs of fusiform shape with small circular ova with a more dense area like a nucleus. When the specimens captured had taken no blood or only a small amount, the ovaries did not undergo development during the next few days.

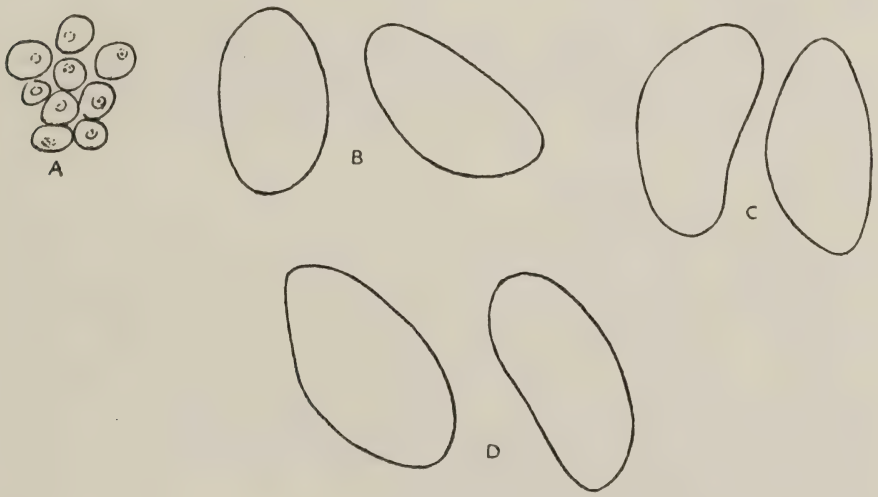

FIG. 4. Ova of *S. damnosum*. A.—Unfed; B.—Fourth day after feed; C.—Fifth day; D.—Seventh day.

* The specimens belong to a new species which will shortly be described by Major E. E. Austen in the *Bulletin of Entomological Research*.

In flies which had fed well the ovarian development was rapid and progressive. The appearance of the ova at various stages is shown in fig. 4. By the fourth or fifth day the ovaries had become partly thoracic in position, so that in separating the thorax from the abdomen a portion of the ovaries and their contained eggs was frequently found in the thoracic region. The increase in size of the ovaries proceeded proportionately to the decrease of blood in the gut so that after a week a fly which had fully fed still had a fully-fed appearance, with this difference, that instead of a globular bulge on each side of the abdominal region, there was a tapering enlargement which diminished as it extended backwards from the thorax to the posterior end of the abdomen.

ACCOUNT OF STAGES

(1) *In subcutaneous nodules.*

The fluid obtained by aspiration of nodules with a syringe contained free larvae, and in several instances where the uterus had been punctured, eggs with embryos in them.

(*a*) *The egg.*

The egg measured from 46μ to 61μ in length, by 33μ to 51μ in breadth ; the egg membrane remained practically unstained when dried, fixed in alcohol and stained with hot haemalum.

(*b*) *The embryo in the egg.*

These measured from 264μ to 290μ in length, by from 7μ to 9μ in breadth ; the cephalic clear area measured from 6μ to 9μ, the caudal clear area from 12μ to 16μ, and the first break from the cephalic extremity was situated at from 21 to 25 per cent. of the length. The nuclei were arranged irregularly in a somewhat scattered manner in the body and were stained to a depth comparable with one group of free larvae in the fluid, but less deeply than another group ; these two groups of larvae occurring in the nodules are referred to below in dealing with the free larvae.

(*c*) *The free larvae.*

The larvae which were found free in the nodule fluid presented two types. The first was a large form very similar, in the scattered

arrangement of the nuclei and in staining reaction, to the form found in the egg ; the measurements were, as a rule, slightly greater than those of the egg forms. The second was a small form having a more compact arrangement of the nuclei and taking the stain more deeply than either the large larvae or the egg forms. Although there was some overlapping in measurements and in the density of staining in individual forms, the differences of the two types were as a rule easily observable.

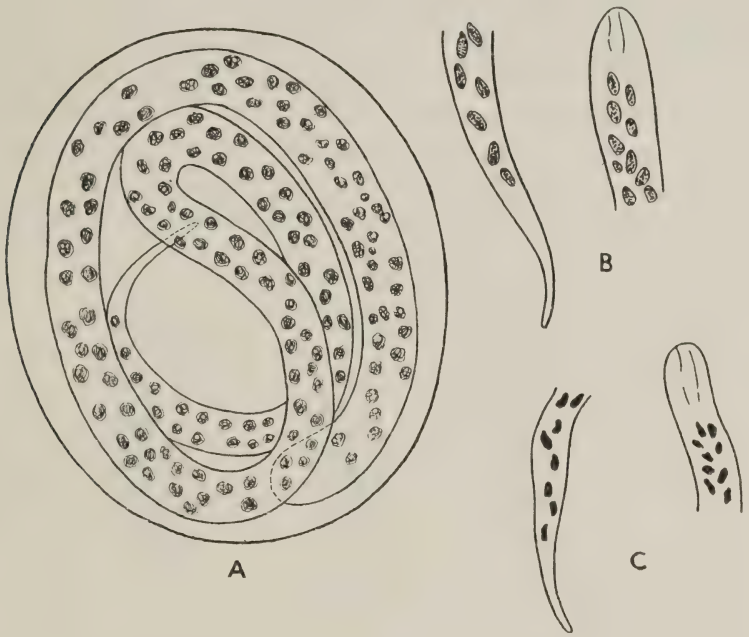

FIG. 5. In puncture fluid from nodule. A.—Larva in egg membrane ; B.—Large pale forms of larva ; C.—Small deeply stained form.

Large forms.

These varied in length from 295μ to 358μ, and in breadth from 6μ to 9μ ; the cephalic clear area measured from 7μ to 11μ and the caudal from 13μ to 18μ ; the first break from the cephalic end was at from 21 to 25 per cent. of the length ; in these forms, as also in the egg forms, owing to the irregular disposition of the nuclei, even the first break was sometimes difficult to determine with accuracy.

Small forms.

These forms usually measured from 221μ to 287μ in length by from 5μ to 7μ in breadth; the cephalic clear area measured from 5μ to 8μ, the caudal clear area from 10μ to 16μ; the first break was situated at from 22 to 25 per cent. of the length.

(2) *In the skin.*

Great variation in size was evident in the forms derived from the skin. Whereas forms which were measured in fresh preparation varied from 306μ to 322μ in length by from 6μ to 9μ in breadth, specimens in water, which had been allowed to dry on the slide, and then fixed by absolute alcohol, and stained, showed remarkable differences in dimensions. The majority were found to measure from 250μ to 300μ in length; in one case, however, one specimen was found of only 184μ in length and several other specimens in the

FIG. 6. *O. volvulus* forms in gut of *S. damnosum*.

neighbourhood of 200μ. The larvae in this case were dissected out in water, allowed to dry on the slide, fixed in absolute alcohol and stained by prolonged immersion in dilute carbol methylene blue followed by heated haemalum. In ten specimens measured in this case, the length varied from 184μ to 229μ; the cephalic clear area measured from 5μ to 9μ and the caudal from 9μ to 21μ; the first break from the cephalic end occurred at from 20 to 23 per cent. of the length.

The usual breadth of the skin larvae was from 5μ to 9μ, the cephalic clear area measured from 5μ to 13μ, the caudal from 9μ to 21μ; the first break from the cephalic end was situated at from 21 to 26 per cent. of the length.

(3) *In the gut of Simulium damnosum.*

The forms found in the gut of the fly measured from 200μ to 334μ in length by from 5μ to 8μ in breadth; the cephalic clear area measured from 6μ to 8μ and the caudal clear area from 10μ to 17μ; the first break from the cephalic end occurred at from 20 to 24 per cent. of the entire length. The measurements are set out in the following table.

Table XI.

Giving the measurements of the forms so far dealt with.

Form	Length μ	Breadth μ	Clear area		First break at per cent. of length
			Head μ	Tail μ	
1. Nodule :—					
(*a*) Egg	46-61	33-51	...	...	...
(*b*) Larvae in egg ...	264-290	7-9	6-9	12-16	21-25
(*c*) Free pale forms ...	295-358	6-9	7-11	13-18	22-25
(*d*) Free dark forms ...	221-287	5-7	5-8	10-16	22-25
2. Larvae in skin	184-322	5-9	5-13	9-21	20-26
3. Larvae in fly gut ...	200-334	5-8	6-8	8-17	20-24

Movements of larvae.

Within the egg in the nodule fluid only sluggish and partial movements of the larvae were observed. The larvae free in the fluid moved actively but the movement was confined to a coiling and twisting movement without progress across the field. The larvae from the skin when freed in a drop of water on a slide were, as a rule, more sluggish in their movements than the free larvae in the nodule fluid. The chief exceptions to this were the larvae obtained in skin sections from the loin in the case of two adult females; the larvae from these cases showed more active movement than did those from the skin or the subcutaneous nodules in the males examined. In the gut of the fly the larvae proved considerably more active in their movements than in either the nodules or the skin; they exhibited even a certain degree of translatory movement when they were in contact with the blood and débris on the slide.

DEVELOPMENTAL FORMS FOUND IN THE THORAX AND HEAD OF *SIMULIUM*

A. In casual flies.

Certain flies dissected soon after capture contained forms of developing larvae in the thorax and head. The developing larval forms were of various sizes and comprised two very distinct stages, with several intermediate forms.

The two stages were:—(1) Forms of a shape corresponding to those given in fig. 7, with a wide body and definite tail, with or without clear spots in the anterior and posterior portions of the body, and with a more or less developed intestine. A series of such forms ranging in length from 166μ to 425μ, and in breadth from 18μ to 43μ, was collected from the dissection of the thorax of casual flies. (2) Forms usually of a greater length than those and devoid of definite tail. These forms were, as a rule, considerably narrower than the preceding and differed in having a definite intestine and a slit-like anus; a series of such forms collected from the dissection of the thorax of casual flies gave a range of length from 560μ to 660μ, and of breadth from 18μ to 28μ.

Developing forms were not often present in large numbers in those casual flies which were infected; in most cases little movement

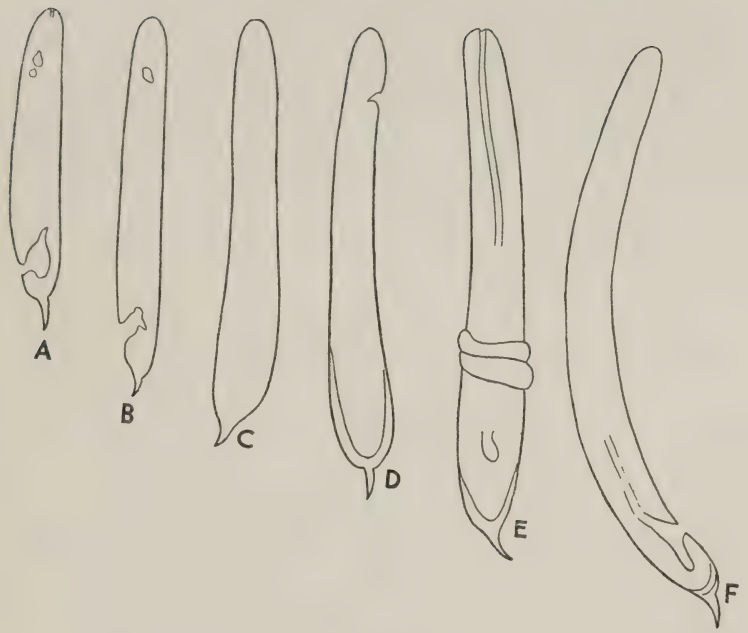

FIG. 7. *O. volvulus* forms with caudal appendage from thorax of wild *Simulium damnosum*. A.—214μ × 34μ; F.—425μ × 38μ. Forms D, E, and F in process of ecdysis.

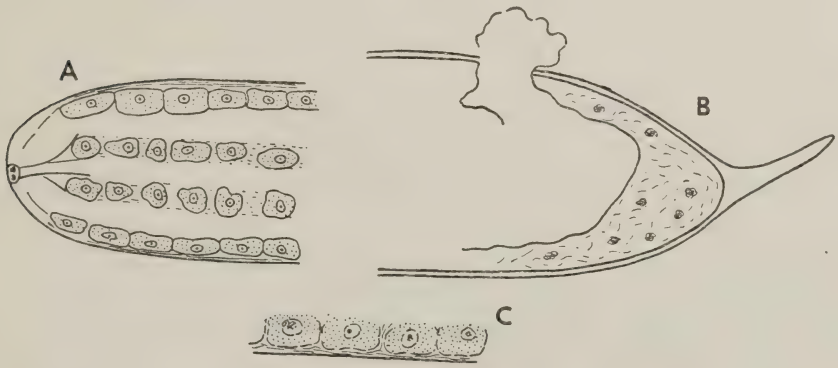

FIG. 7A. *O. volvulus* forms in thorax, wild *Simulium damnosum*, 266μ × 33μ. A.—Head end; B.—Tail end; C.—Subcuticular cell layer, mid-third of body.

was observed beyond a slight flexion of one or other extremity. Some of the large forms, however, continued to move for a short time after liberation from the thorax. The only form which exhibited very active and prolonged movement came out of the thorax attached to the head on separation of the head from the thorax. It moved very actively and incessantly for a period of three hours, showing very considerable power of penetrating masses of débris on the slide. Unfortunately it was lost in the manipulation of the coverslip. This individual is not included in the measurements given.

B. In flies kept alive after feeding on infected skin.

The forms found in these flies at various dates after being captured feeding on infected human skin were similar in measurements and in morphology to those found in the flies of group *A*. A series of wide forms possessing a tail varied in length from 173μ to 461μ, and in width from 17μ to 45μ ; a series of forms without a tail measured from 349μ to 628μ in length, and from 20μ to 38μ in width.

Some of the longer forms exhibited active movements, but it was observed that some forms which were active were of smaller size than other inactive forms in the thorax of the same fly. This possibly has a connection with the process of ecdysis.

The numbers of forms dissected out of the thorax in several of the flies which had thus fed on known infected skin was large. In a fly which was dissected sixty hours after thus feeding, no less than 236 developmental forms were recovered from the thorax. In another fly which died on the fifth day sixty forms were found. In a fly which lived until the seventh day ten forms of a much more advanced stage of development were found. It was observed that in some flies there were present forms of different stages. The explanation which naturally suggests itself is that those flies had acquired infection at some period previous to, as well as at the time of, their feeding on the infected case. Such an explanation will not, however, suffice to account for a case such as the following. The flies referred to above which had fed sixty hours previously on infected skin presented, in addition to the early thoracic developing forms, a form which appeared to be at the same stage of development

as the forms just ingested by the fly, and a form of shorter and broader type evidently about midway in development. It was in excellent condition and stained well. The explanation of this probably is either that some of the forms ingested by the fly have not developed sufficiently before their ingestion to enable them to keep pace with the rest, or that they have for some reason failed to reach a suitable portion in which further development can proceed.

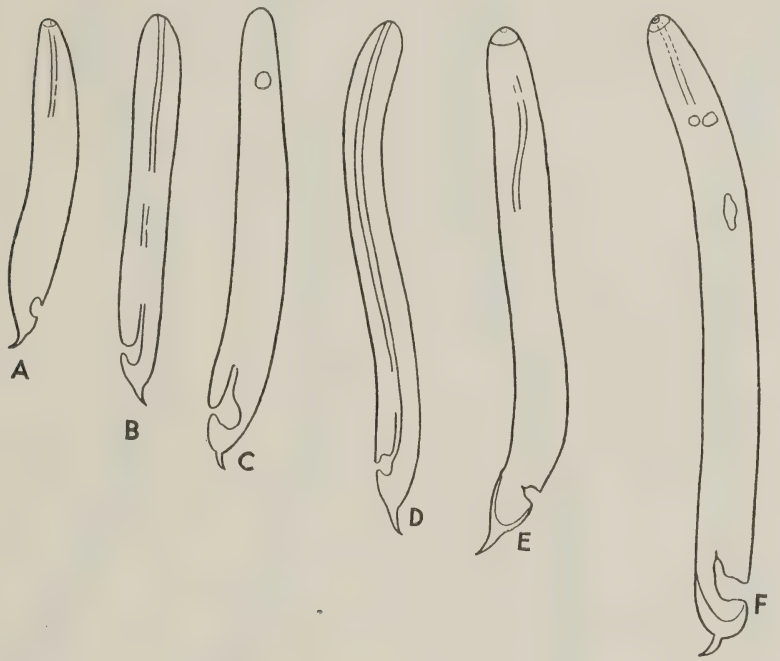

FIG. 8. *O. volvulus* forms with caudal appendage from thorax of *S. damnosum*, five days after feeding on infected skin. A.—223μ × 36μ; F.—440μ × 36μ. Forms E and F in process of ecdysis.

The argument which is applicable to this case can probably, with justification, be applied to cases at a later date where, in the same fly presumably infected at the time of feeding on known infected skin, there appear, in addition to forms which represent a correct stage of development for the period as judged by the average development in other flies, forms which represent an earlier stage.

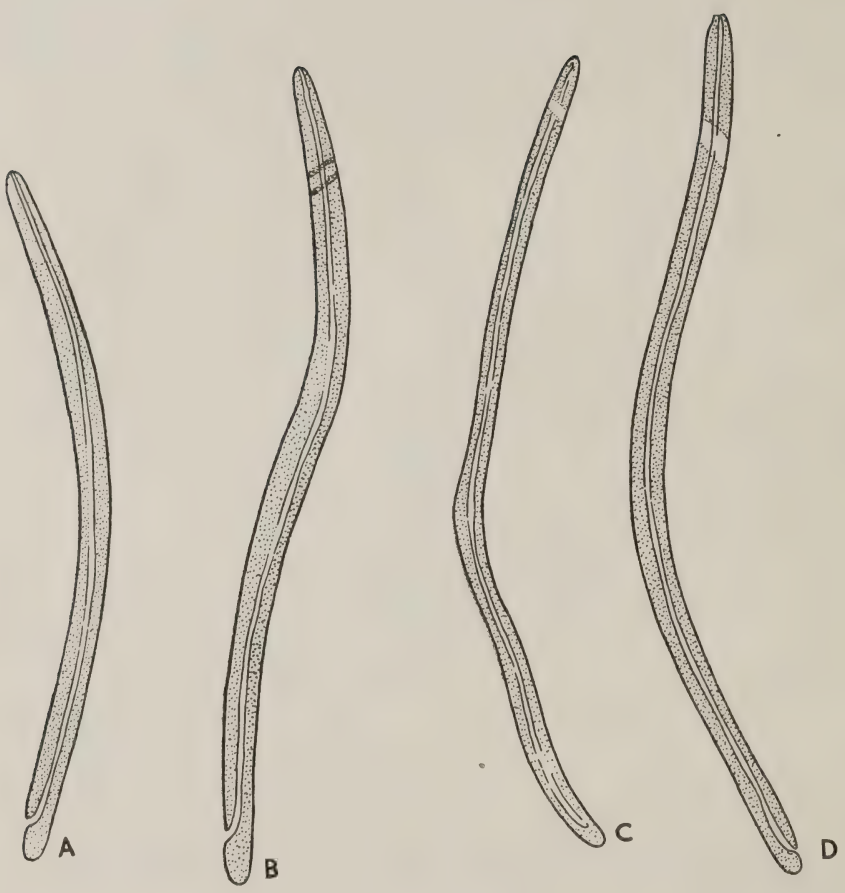

FIG. 9. A.—*O. volvulus* form from thorax of *S. damnosum*, seven days after feed. 505μ × 25μ.
B.—*O. volvulus* form from thorax of *S. damnosum*, seven days after feed. 592μ × 32μ.
C.—*O. volvulus* form from thorax of *S. damnosum*, wild, seven days after feed. 616μ × 25μ.
D.—*O. volvulus* form from thorax of *S. damnosum*, wild, seven days after feed. 657μ × 28μ.

Ecydsis.

It is evident that throughout the process of development of the larva several changes are brought about which could most readily be explained on the basis of ecdysis. If this is not the explanation it is difficult to understand how, at various stages, the larva undergoes the considerable variations in length and breadth observed. Thus in the tumour fluid the large pale form which resembles the egg form is associated with a smaller and darker-stained form with compact nuclei ; in the skin, only forms corresponding to the latter type were found, but again with a diminution in length of some individuals. In the thorax the shortest form found in any fly measured only

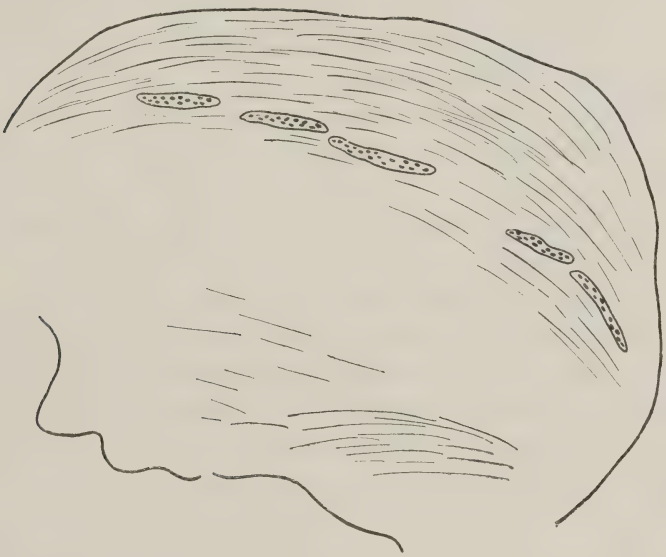

Fig. 10. Section of thorax of *S. damnosum* on fifth day after feeding on infected skin, showing portions of developing forms of *O. volvulus* larvae.

166μ, yet in the skin of infected persons the shortest form found was 184μ, while in the gut of infected flies the shortest form found was 200μ. What seems to be actual ecdysis was only observed in the case of the transition from the tailed thorax form to the thoracic form without tail ; it was observed that in some cases the active forms later developed in the thorax were accompanied by forms slightly longer and immobile, and the appearances suggested that at this stage also, an ecdysis had occurred from the more elongated immobile form to the active form.

SUMMARY

1. *Onchocerca volvulus* infection is common in the Konno District of the Protectorate of Sierra Leone ; larvae of this parasite were found in the skin of 45 per cent. of persons examined systematically.

2. A definite relationship between diseases of the skin or diseases of the eyes and the infection with *O. volvulus* as judged by the presence of larvae in the skin could not be established.

3. *Simulium damnosum* is very prevalent in the hilly country, which is covered with bush and grass, and has numerous streams and rivers ; the lesion inflicted on the skin by this species in biting is such as would dislodge the larvae of *O. volvulus* in the skin.

4. Dissection of wild insects showed :—

(*a*) In 780 dissections of the gut an infection of 2·6 per cent. with larvae morphologically identical with those of *O. volvulus*.

(*b*) In 1,320 dissections of the thorax a larval infection of over 1 per cent.

5. By allowing wild flies to feed on restricted and heavily-infected areas of the skin the gut infection was raised to 80 per cent. in one experiment and the thorax infection to nearly 82 per cent. in another experiment ; the developing forms of *O. volvulus* were found in the thorax after the infecting feed up to the seventh, eighth, and tenth days, after which period no insect survived.

EXPLANATION OF PLATE I

Fig. 1. Nodules on both trochanters.

Fig. 2. Nodules on ribs (1), and trochanter (2). Puncture positive in both.

Fig. 3. Painful nodules and a much wrinkled skin.

Fig. 4. Case 59. Nodules on right elbow (1), and right trochanter (2).

1.

2.

3.

4.

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EXPLANATION OF PLATE II

Fig. 1. Case 59. Nodules on left elbow (1), left trochanter (2), and left knee (1).

Fig. 2. Case 59. Nodules (near view, right side) on elbow (1), and trochanter (2.)

Fig. 3. Capturing *S. damnosum.*

Fig. 4. Type of country infested by *S. damnosum.*

1.

2.

3.

4.

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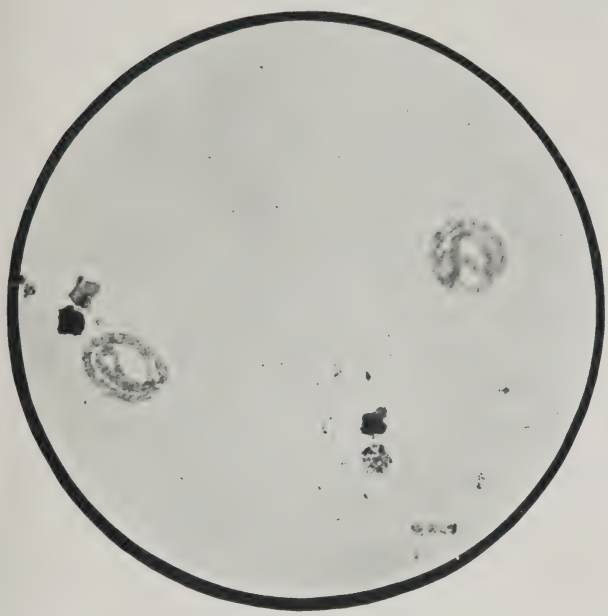
EXPLANATION OF PLATE III.

Fig. 1. Micro-photograph showing two eggs of *O. volvulus* in puncture fluid from a subcutaneous nodule. × about 200.

Fig. 2. Micro-photograph of larva of *O. volvulus* in puncture fluid from a subcutaneous nodule. × 200.

Fig. 3. Micro-photograph of two larvae of *O. volvulus* from skin. × 200.

Fig. 4. Micro-photograph of larvae of *O. volvulus* from gut of *S. damnosum*. × 200.



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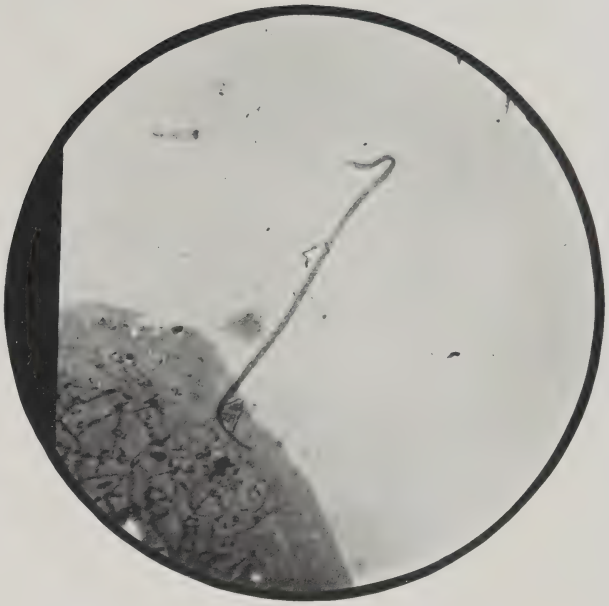

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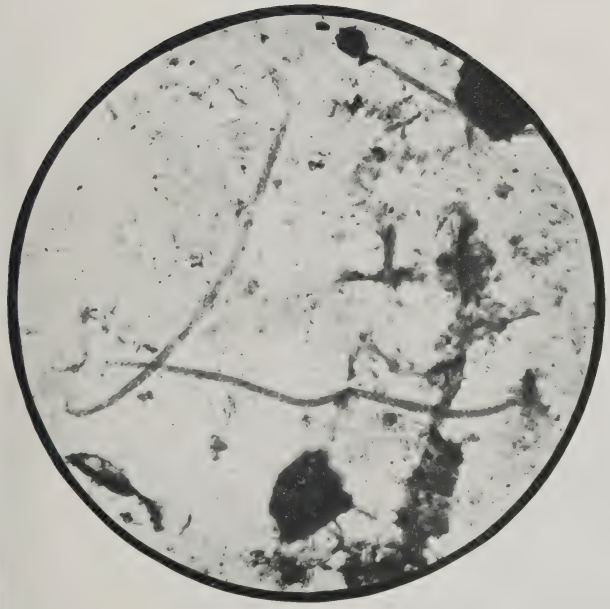

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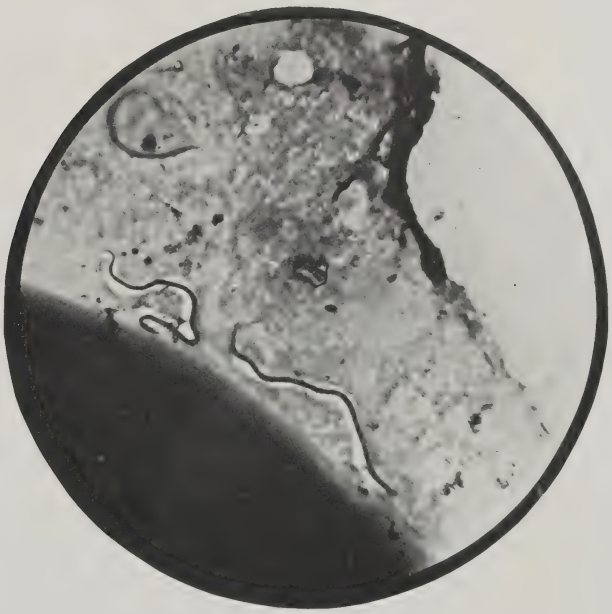

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EXPLANATION OF PLATE IV

Fig. 1. Micro-photograph of larva of *O. volvulus* from thorax of *S. damnosum* sixty hours after ingestion. × 200.

Fig. 2. Micro-photograph of two larvae of *O. volvulus* from thorax of *S. damnosum* seven days after ingestion. × 125.

Fig. 3. Micro-photograph of section of skin showing larvae of *O. volvulus*. × 200.

Fig. 4. Micro-photograph showing skin lesion produced by the bite of *S. damnosum*. × 20.

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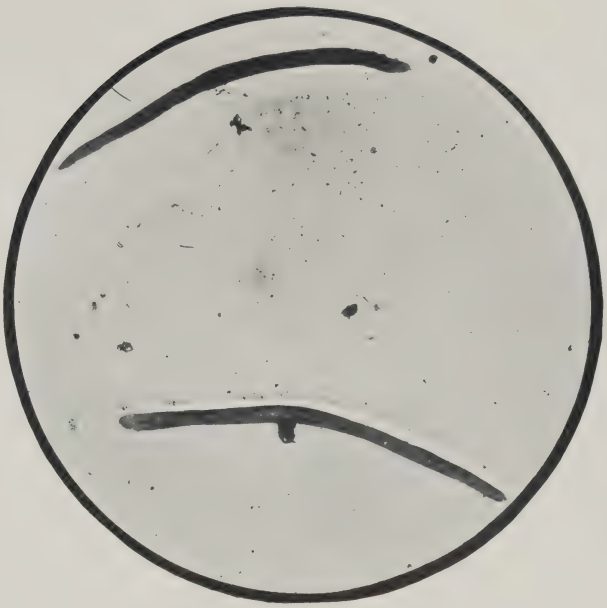

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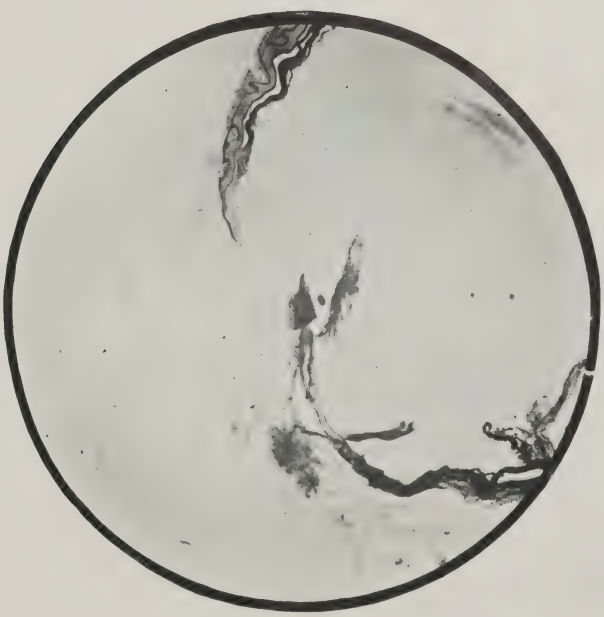

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